PARALYTIC SHELLFISH POISONING:

ITS HISTORY, PROCESSES AND IMPACTS

AS APPLICABLE TO PUGET SOUND

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ABSTRACT

This document contains the initial report of the Paralytic Shellfish Poisoning (PSP) Task Group. The report provides a synopsis of available information concerning the history, processes, and impacts associated with PSP as applicable to Puget Sound.

PSP outbreaks appear to be spreading to previously unaffected areas and are increasing in intensity worldwide as well as locally. This report includes a review of this trend and of the current toxicity monitoring programs established to protect the public from PSP. Attention is also given to possible causal factors associated with toxic dinoflagellate blooms, particularly dinoflagellate cysts and certain environmental factors such as temperature, organic chelators, trace metals, and precipitation.

Recent studies suggest that; 1) man is not the only organism susceptible to dinoflagellate toxins; and 2) the toxins may be transferred to higher trophic levels by organisms other than shellfish. The report discusses these findings as well as the nature of dinoflagellate toxins and their effects on man.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the willing assistance and interest of Martha Simon and the Department of Social and Health Services for providing current shellfish toxicity data, and an explanation of the local toxicity monitoring program. We also thank Bonnie Mattson and the Metro library staff for their efforts in conducting the literature search critical to the gathering of information for this report. Dr. Kenneth Chew, Louisa Nishitani and John Wakeman of the University of Washington's School of Fisheries, and John W. Hurst, Director of Resource Services for the Maine Department of Marine Resources, Fisheries Research laboratory are also acknowledged for their invaluable assistance and support.

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SUMMARY

This report is a synthesis of the information reviewed to date by the Paralytic Shellfish Poisoning (PSP) Task Group since its inception in July, 1981. The report summarizes the history, processes, and impacts associated with PSP as applicable to Puget Sound.

PSP is a form of food poisoning in which extremely lethal toxins, produced by certain dinoflagellates, are accumulated in shellfish and passed on to humans. The threat of shellfish toxicity has been recognized for centuries; however, in recent years, PSP has spread to previously unaffected areas throughout the world, and the outbreaks appear to be increasing in intensity.

This trend has become apparent locally in recent years with the spread of PSP into the central Puget Sound basin. As a result, most beaches north of the Tacoma Narrows have been closed to the harvesting of shellfish for much of 1980 and 1981.

Toxicity monitoring programs have been established by local public health officials in most regions supporting shellfisheries. Closures are issued when results of the mouse bioassay indicate that the toxicity of shellfish in a particular area equals, or exceeds, 80 ug/100 grams of shellfish tissue. At present, the location and frequency of sample collection is varying considerably due to the effects of funding and manpower limitations on local health departments. An example locally is the closure of all of King County beaches from October, 1981 through February, 1982, based on high toxicity levels in shellfish taken from Alki and Fauntleroy on October 12, 1981.

A close correlation exists between the level of toxin in shellfish and the concentration of toxic dinoflagellate cells within a given water body. Recent studies indicate that the initiation of "blooms" in the dinoflagellate population may be associated with the germination of dinoflagellate cysts -- a dormant form which may be assumed by the dinoflagellates when environmental factors become unfavorable.

Although numerous environmental factors apparently influence development of a bloom from newly emergent germlings, the introduction of certain organic compounds to coastal waters may create an environment favoring growth of the dinoflagellate population by controlling the availability of certain growth-regulating trace metals. These compounds, called chelators, may be introduced to the marine environment naturally by runoff associated with precipitation events, or in association with urban development.

The spread of PSP may be associated with the transport of toxic dinoflagellate cysts by currents, dredging and other construction activities, and possibly by other marine organisms. The fact that motile cells and cysts of the locally occurring toxic dinoflagellate, Gonyaulax catenella, have been found at various Puget Sound locations, suggests that the potential exists for PSP outbreaks throughout the sound.

Serious implications are raised by the recent discovery that dinoflagellate toxins may be lethal to organisms other than man. For example, consumption of toxic shellfish may prove fatal to certain species of birds. In addition, recent investigations indicate that dinoflagellate toxins can be accumulated and retained by herbivorous zooplankton feeding on toxic dinoflagellates, and then passed on, in lethal levels, to fish consuming the zooplankton.

SECTION I

INTRODUCTION

Paralytic Shellfish Poisoning (PSP) is a form of food poisoning in which toxins, produced by certain species of dinoflagellates, are accumulated in shellfish and passed on to humans consuming the shellfish. Although toxin-producing dinoflagellates have been observed in low numbers in the central Puget Sound basin, the first outbreak of toxic conditions did not occur until 1978. Since that time, PSP outbreaks have spread throughout most of Puget Sound and appear to be increasing in intensity. To remain abreast with current research and developments relating to PSP, Metro established the PSP Task Group in July, 1981. This report is a compilation of the information reviewed by the task group to date.

HISTORY OF PARALYTIC SHELLFISH POISONING (PSP)

Consumption of shellfish has been associated with occasional illness and death throughout recorded history. At least 300 fatalities have been reported worldwide as a result of PSP (Dale and Yentsch, 1978). Four major coastal regions, the east and west coasts of North America, the western European coastline, and the Japanese islands, have been significantly impacted by annual outbreaks of shellfish toxicity. In addition, PSP incidents have recently been reported along the West coasts of South America and Africa, in the Red Sea, and along the Australian, Indonesian, and New Zealand coasts (Dale and Yentsch, 1978; Hockey and Cooper, 1980; Ballentine, 1981).

Toxic dinoflagellates of the genus Gonyaulax have been identified as the causal organisms in the majority of paralytic shellfish poisonings. This genus includes the armored dinoflagellates

responsible for PSP incidents along the West coast of North America from Alaska to California, and along the East coast from the Bay of Fundy to Rhode Island. The species G. tamarensis has been identified as the causal organism on the East coast of North America (Anderson and Wall, 1978; Collins, 1978; Anderson and Morel, 1979). In addition to causing toxicity in shellfish, this species has also been linked with recurrent Atlantic herring kills (White, 1980; White, 1981).

Gonyaulax catenella is the toxic dinoflagellate responsible for shellfish toxicity along the West coast of North America. This species has a long history of extreme toxicity. Recorded incidents date back to 1793, when one of Capt. George Vancouver's men died after eating toxic mussels from the northern British Columbia coast; and to 1799, when 100 Russian hunters died in Sitka, Alaska less than two hours after consuming toxic mussels (Seattle times, 6-27-81; Ballentine, 1981).

PARALYTIC SHELLFISH POISONING IN PUGET SOUND

History

Historically, G. catenella has been a hazard along the open coast of Washington, British Columbia, Alaska, and the Straits of Juan de Fuca, but not within Puget Sound. In 1975, however, commercial shellfish operations near Bellingham were closed as a result of excessive shellfish toxicity (Nishitani, personal communication). This was followed by a major bloom of G. catenella at Penn Cove on Whidbey Island in 1978. Shellfish toxicity levels at Penn Cove were as high as any previously recorded along the West coast. Shellfish toxicity was not confined to Whidbey Island, however, as traces of toxin were reported as far south as Des Moines (Nishitani, personal communication; Seattle Times, 6-27-81).

The spread of PSP south into Puget Sound has continued during the past three years. Toxins were found at the Tacoma Narrows in 1979, near the mouth of the Nisqually River in 1980, and in Budd and Totten Inlets at the south end of Puget Sound in 1981 (Nishitani, personal communication; Seattle Times, 6-27-81). Toxin levels south of the Tacoma Narrows have not exceeded the level requiring closure of beaches to the harvesting of shellfish (see Figure A-1). Beaches north of the Narrows, however, have been closed for much of 1980 and 1981 due to unsafe levels of PSP in shellfish (see Figure A-2) (Simon, personal communication; Nishitani, personal communication).

Possible Cause for Spread of PSP in Puget Sound

G. catenella cysts, a dormant form assumed by the dinoflagellates when environmental conditions become unfavorable, may be partly responsible for the spread of PSP into Puget Sound. Cysts apparently behave similar to fine sediment particles, and may be transported to previously unaffected areas by currents, dredging and other construction activities, and possibly by other marine organisms (Dale, Yentsch and Hurst, 1978; Seattle Times, 6-27-81). Although little information exists regarding the possible role that cysts might play in the spreading of PSP, the fact that cysts have recently been found in bottom sediments at various Puget Sound locations suggests that the potential exists for PSP outbreaks throughout the Puget Sound area (Nishitani, personal communication).

SECTION II

DYNAMICS OF TOXIC DINOFLAGELLATE BLOOMS

With the spread of PSP into previously unaffected areas has come an increased interest in the dynamics of toxic dinoflagellate blooms. It has been recognized for some time that a close correlation exists between toxin levels in shellfish and the concentration of motile, toxic dinoflagellate cells within a given water body. Motile cells often form long chains of cells, and are characterized by a whip-like tail called a flagellum which provides a limited mechanism for propulsion; and by an encircling flagellum, or girdle, which acts as a rudder or steering mechanism. During periods when environmental conditions favor growth, usually between April and October, the concentration of motile cells may increase very rapidly, or "bloom". Researchers at the University of Washington have recently determined that, under optimum conditions, the motile cells may divide at a rate as high as one division per day (Nishitani and Wakeman, personal communication).

POSSIBLE ADAPTIVE CAPABILITIES OF TOXIC DINOFLAGELLATES

Although it has generally been thought that the genus *Gonyaulax* is primarily autotrophic organisms requiring light for photosynthesis to sustain life and reproductive functions, a recent study discovered that *G. polyedra* is apparently capable of heterotrophic behavior utilizing available nutrients to support growth and reproduction. Because of the similarity between *G. polyedra*, generally a non-toxic dinoflagellate, and *G. catenella*, it is possible that the latter may also possess the ability to utilize alternative food sources (Hudgins, 1981; Kleppel, personal communication). Additional research is required, however, before such adaptive behavior can be confirmed and the implications fully assessed.

Another example of the possible adaptive capabilities of toxic dinoflagellates is the observation that motile cells may enter one of two types of encystment stages when environmental conditions are not conducive to growth, dormancy, or an adaptation to short-term adversities.

Dormancy

One type of possible adaptive capability is associated with extended periods of dormancy, or overwintering, and may be a zygote produced in sexual reproduction. This type is characterized by thick-walled, capsule-shaped cells termed hypnocysts, or resting cysts, which settle out of the water column and accumulate in the upper layer of sediment. The following spring, when conditions again become favorable, the hypnocysts may germinate and return to the motile form (Anderson and Morel, 1979; Anderson and Wall, 1978; Dale and Yentsch, 1978).

Adaptation to Short-Termed Adversities

The second type of encystment is apparently an adaptation to short-term adversities. Cysts of this type are thin-walled, rounded, non-motile cells termed pellicle, or temporary cysts, which seem capable of only limited periods of encystment. This type of cyst is thought to be asexual (Anderson and Wall, 1978). Pellicle cysts apparently remain suspended within the water column, and when studied in culture, have shown the ability to excyst and establish a motile population within hours of conditions becoming favorable (Anderson and Morel, 1979; Anderson and Wall, 1978; Dale and Yentsch, 1978).

CAUSAL FACTORS ASSOCIATED WITH TOXIC DINOFLAGELLATE BLOOMS

Recent investigations concerning possible causal factors associated with blooms of _G. tamarensis_ have indicated that a close correlation exists between seasonal variations in water temperature and germination of hypnocysts. Laboratory studies, as well

as field observations, indicate that hypnocyst germination is likely to occur with a high rate of success when the hypnocysts have been exposed to extended periods of cold temperatures, followed by gradual warming. Once germination has been initiated, excystment apparently will continue regardless of other external factors (Anderson and Morel, 1979; Anderson and Wall 1978; Dale and Yentsch, 1978).

Bloom Development

Specific environmental factors, such as temperature, salinity, trace metals, nutrients and other organic compounds, light intensity, photoperiod and hydrographic mechanisms, must create a suitable environment in order for a bloom to develop from a seed population of newly emergent germlings (Anderson and Morel, 1979; Dale and Yentsch, 1978). These factors may often be influenced by meterological events such as wind and precipitation.

Precipitation

Several studies have noted that toxic dinoflagellate blooms tend to occur along the coasts following significant precipitation. This may be especially noteworthy with regards to Puget Sound, given that the 1978 Whidby Island bloom was preceded by record levels of precipitation and runoff. Recent studies suggest that organic compounds which may influence bloom development can be introduced to the marine environment through natural processes, or in association with urban development.

Organic Compounds and Trace Metals

Existing evidence suggests that certain organic compounds and trace metals may regulate the growth and distribution of toxic dinoflagellates. With an increase in organic compounds, for example, there would also be a greater availability of iron. Because dinoflagellates require larger quantities of iron than most phytoplankters, this could give toxic dinoflagellates a com-

petitive advantage over other algal species (Dale and Yentsch, 1978). Conversely, laboratory experiments have shown that certain species of Gonyaulax will prosper only in a growth medium with a very low cupric ion concentration. The introduction of certain organic compounds, termed chelators, would likely result in the cupric ions being tied up within larger molecules, and thereby lower the concentration of available copper (Anderson and Morel, 1978; Dale and Yentsch, 1978; Yentsch and Glover, 1978).

Bloom Decline

As with the initiation of toxic dinoflagellate blooms, a number of possible interrelated factors apparently influence the decline of a bloom. Changes in the external environment, which are not conducive to growth and reproduction, will usually result in a decline of the dinoflagellate population. Such changes may include variations in wind, current or advection patterns, fluctuations in temperature, nutrient availability or trace metal concentrations, or increased predation. It should also be noted that parasitism has tentatively been linked to the decline of a G. catenella bloom in Quartermaster Harbor (Vashon Island) during 1981 by researchers at the University of Washington (Nishitani, personal communication).

Parasitism

Although parasitism could prove a significant factor in explaining the decline of toxic dinoflagellate blooms, it might also provide a mechanism for the biological control of dinoflagellate populations. The parasite Amoebophrya ceratii, observed during the University of Washington studies, apparently infested G. catenella cells and destroyed not only the nucleus of the host cell, but also the nuclei of adjacent cells in the chains. The parasite produced spores which grew within the host cell and eventually caused the old dinoflagellate cell wall to

rupture, releasing the spores. The rate of infestation apparently was much more rapid than the fastest growth rates observed for *G. catenella* (Nishitani and Wakeman, personal communication).

Researchers at the University of Washington suggested that non-toxic dinoflagellates might be artificially infested with the parasite, and then be introduced in the vicinity of a toxic bloom in hopes of controlling the toxic dinoflagellate population. As feasible as this approach might appear for controlling PSP outbreaks, much additional research would be required before this type of biological control could be implemented. At present, however, research at the University of Washington on toxic dinoflagellate blooms has been curtailed due to a lack of funding (Nishitani and Chew, personal communication).

SECTION III

TOXICITY MONITORING PROGRAMS

To protect the public from the threat of PSP, State of Washington health officials have established toxicity monitoring programs in most areas which support commercial and/or sport shellfisheries. Shellfish collected from various shorelines are tested for toxicity using the standard mouse bioassay. This procedure, developed in 1937, involves macerating shellfish tissue and injecting laboratory mice with the liquid extract. The time from injection until the mouse's death is used to calculate the toxin level. Beach closures are ordered when shellfish toxicity levels equals, or exceeds, 80 ug/100 g of shellfish tissue (Simon, personal communication; Seattle Times, 6-27-81; Dale and Yentsch, 1978).

SAMPLE COLLECTION

With the exception of commercial shellfish operations, which submit samples for toxin analysis at a given frequency, collection of shellfish from public beaches for testing is the responsibility of local public health departments. With the present restrictions on funding and manpower, local health departments are having to rely more and more on shellfish samples being collected and submitted by the general public. Consequently, the location and frequency of collection often varies greatly (Simon, personal communication; Lilja, personal communication).

An example of this locally is represented by the lack of toxicity data for King County beaches during the months of October, 1981 through January, 1982. Mussels collected from Alki Point and the Fauntleroy ferry dock on October 12, 1981 had reported toxicity levels of 311 and 412 ug/100 g tissue, respectively (Simon, personal communication). As of early February, 1982, when this

report was drafted, beaches within King County were still closed on the basis of the October, 1981 toxicity data.

BEACH CLOSURES

Beach closures often result in financial loss to the seafood industry. The following list of coastline closures may serve to exemplify the extent of such impacts:

- Since 1937, the entire coast of California has been closed annually between May 1, and October 31, to the harvesting of mussels (Hudgins, 1981).

- Since 1942, the Washington coast from Dungeness spit to the Columbia River has been closed annually between April 1 and October 31 to the harvesting of clams (excepting razor clams) (Nishitani, personal communication; Seattle Times, 6-27-81 and 10-18-81).

- Since 1947, the coast of Alaska has been closed year round to the harvesting of clams and mussels (Dale and Yentsch, 1978).

- The closure of the Maine coast to the harvesting of shellfish from late August to late October, 1980 resulted in estimated losses to the shellfish industry in excess of seven million dollars (Ballentine, 1981).

ALTERNATE TESTING TECHNIQUES

Other strategies are currently being investigated which could possibly replace the mouse bioassay technique as the basis for determining beach closures. Several attempts have been made to develop an index of critical environmental factors which could be used to predict the location and timing of toxic dinoflagellate blooms. However, predictive indices developed to date have not

proven to be reliable; largely due to the number of interrelated environmental factors which may be involved with bloom initiation and development. Attempts to develop a reliable chemical assay for determining toxin levels has also met with little success to date, as a result of the complex molecular structure of paralytic shellfish toxins (Ballentine, 1981; Dale and Yentsch, 1978; Simon, personal communication).

SECTION IV

DINOFLAGELLATE TOXINS

Certain dinoflagellates, belonging to the genus Gonyaulax, metabolize toxins which are collectively called Gonyaulax toxins (Ballentine, 1981). One of these toxins, saxitoxin, is an extremely lethal poison for which there is no known antidote. Gonyaulax toxins are bound in the tissue of shellfish that feed upon toxic dinoflagellates without harming the shellfish. The binding is not extremely strong, however, as the toxins are released in a weak acid solution (Collins, 1978). Apparently, strong acids in the human stomach will rapidly release the bound toxins, thereby causing PSP.

Gonyaulax toxins are nitrogeneous hydrochlorides which affect the sodium/potassium balance, which controls electrical conduction in the nerves, thereby depressing both peripheral nerves and reflex transmissions in humans (Dale and Yentsch, 1978). These toxins manifest themselves with the following symptoms:

- Numbness of lips, tongue, and finger-tips within minutes after consumption.
- Numbness in the legs, arms, and neck with general muscular incoordination.
- A feeling of floating, dizziness, weakness, and incoherence.
- Respiratory distress and muscular paralysis that becomes progressively more severe.
- Death results from respiratory paralysis within 2 to 13 hours.

When toxin levels are very high, death may result from eating as little as half a clam. However, if one survives 24 hours, complete recovery is assured with no lasting effects (Dale and Yentsch, 1978; Quayle, 1969).

Varying levels of toxicity have been reported as causing illness or death in humans. For example, one study reported that ingestion of 125 to 190 ug of toxin can result in illness, and that death may occur after ingesting 450 to 1,100 ug (Ballentine, 1981). Another study reported that it takes a total consumed toxin level of 1,000 ug to produce illness in humans, with 10,000 ug being a lethal level (Dale and Yentsch, 1978). In either case, a total consumed level of 1,000 ug would have serious consequences to human health. A level of 1,000 ug could be attained either by ingesting 12 clams, weighing 100 g each with a toxin level of 80 ug/100 g tissue, or by ingesting one clam weighing 100 g with a toxin level of 1,000 ug/100 g tissue (Dale and Yentsch, 1978).

ABILITY TO ACCUMULATE PARALYTIC TOXIN

The ability of shellfish to accumulate paralytic toxin is determined by various factors:

- The concentration of Gonyaulax sp. cells in water.
  A concentration as low as 10 cells/ml can result in toxicity levels in shellfish of 80 ug/100 g tissue (Nishitani, personal communication). However, during peak blooms, toxicity levels exceeding 20,000 ug/100 g tissue have been recorded (Ballentine, 1981; Dale and Yentsch, 1978; Quayle, 1969).

- The species of shellfish.
  Under optimal conditions, the soft shelled clam, *Mya arenaria*, is capable of increasing toxicity at a rate of 116 ug/100 g tissue in 24 hours, while under the

same conditions, the blue mussel, Mytilus edulis, can increase its toxicity as much as 500 ug/100 g tissue in 24 hours (Hurst and Gilfillan, 1977).

- The shellfish's food source.
  Shellfish that feed upon Gonyaulax sp. cysts will accumulate toxin faster than shellfish that feed on the motile form (Dale, Hurst and Yentsch, 1978). Encysted cells of Gonyaulax sp. have been found to be at least 10 times more toxic than motile cells. Some of the literature suggests that the toxicity of encysted cells, when first formed, may be up to 1,000 times more toxic than motile cells, but that over time, the toxicity decreases (Dale and Yentsch, 1978).

- Winter toxicity.
  High levels of PSP during the winter months have been a recent occurence in Puget Sound. Since the motile form of Gonyaulax sp. does not prosper during the colder winter temperatures, the toxicity levels must be attributed to some other causal factor. Early winter toxicity levels could be the result of winter cooling, which slows the depuration of toxin due to reduced physiological activity of the shellfish (Hurst and Yentsch, 1981; Hurst and Gilfillan, 1978). Extended periods of high toxicity levels during the winter months may be the result of shellfish feeding on the extremely toxic encysted form of Gonyaulax sp. (Hurst and Yentsch, 1981; Dale, Hurst and Yentsch, 1978).

RATE FOR DECREASING TOXIN LEVELS

The rate at which shellfish can decrease toxin levels, once Gonyaulax sp. are not part of the food source, is determined

by the species of the mollusc. Studies on the East coast have indicated that the soft shelled clam (Mya arenaria) can decrease its toxicity by 7 to 8 percent a day, lowering a 1,000 ug/100 g tissue level to below 80 ug/100 g tissue level in about thirty days (Hurst and Gilfillan, 1977). Similarly, researchers at the University of Washington have observed that the blue mussel (Mytilus edulis) is capable of a 56 percent reduction in toxin level in seven days (Nishitani, personal communication). However, butter clams (Saxidomus giganteus) and surf clams (Spinsula solidissima) normally remain toxic for a much longer period of time (Hurst and Gilfillan, 1977). In butter clams, a close relationship exists between the anatomical distribution of melanin and the distribution of paralytic toxin. Melanin may play a cation exchange role in the retention of paralytic toxin by these clams (Neve', 1979).

CHEMICALLY INACTIVATING TOXIN

Several methods of chemically inactivating paralytic toxin have been attempted. However, most of the procedures developed have been impractical for the detoxification of live shellfish, as the adverse affects of the chemicals used can destroy the marketability and economic value of the shellfish (Blogoslawski, et al., 1979).

OZONIZED SEAWATER TREATMENT

Submerging clams in ozonized seawater has been the most effective method developed to detoxify contaminated shellfish. Under laboratory conditions, toxicity levels less than 260 ug/100 g tissue have been inactivated by ozone gas treatment with no physiological or histological damage to the shellfish. However, the optimal ozone dosage and exposure time to totally detoxify paralytic toxin has not been determined. The use of ozone gas to inactivate paralytic shellfish toxin has received little attention by commercial shellfish growers in the U.S., while in Europe, ozone gas has been used for years to cleanse bacterially contaminated shellfish (Blogoslawski, et al., 1979).

SECTION V

TRANSFER OF TOXIN TO HIGHER TROPHIC LEVELS

LOWER ORGANISM SUSCEPTIBILITY

It should be noted that man is not the only organism susceptible to the transfer of dinoflagellate toxins through shellfish. PSP outbreaks on the East coast of North America, in England, and in South Africa have been fatal to various species of birds which feed on shellfish (Ballentine, 1981; Hockey and Cooper, 1980). The dinoflagellate responsible for the mortality of Black Oystercatchers, Southern Blackbacked gulls, and Hartlaub's gulls in South Africa has been identified as *Gonyaulax catenella* -- the same species responsible for PSP in Puget Sound (Hockey and Cooper, 1980).

In addition to the mechanism by which dinoflagellate toxins are transmitted by shellfish, several other paths may exist for the transmission of these toxins to higher trophic levels. For example, fish kills associated with *Gymnodinium breve* blooms apparently result from respiratory paralysis caused by toxins released when the unarmored cells rupture against the gills of fish passing through the blooms (Taft, personal communication).

ALTERNATE PATHS OF TOXIN TRANSFER

Blooms of the armored dinoflagellate *Gonyaulax tamarensis* has also been linked to fish kills, specifically Atlantic herring and sand lances. It's believed, however, that the mechanism for toxin transfer in this case is much more complex than with the unarmored dinoflagellates. Recent investigations indicate that herbivorous zooplankton feeding on toxic dinoflagellates can accumulate and retain the toxins at levels which can be lethal to fish consuming the zooplankton (White, 1981, 1980).

It has yet to be determined whether herbivorous zooplankton are also capable of vectoring toxins produced by Gonyaulax catenella to higher marine organisms; however, given the similarities between G. tamarensis and G. catenella, it is probable that such transfer mechanisms exist. If true, this could have serious implications concerning the possibility of fish kills along the Pacific Coast and within Puget Sound, as a result of toxic dinoflagellate blooms.

QUESTIONS FOR FUTURE INVESTIGATIONS

Other questions regarding the transfer of dinoflagellate toxins which need further investigation are: Whether additional mechanisms exist for the transfer of dinoflagellate toxins between trophic levels; and, What are the implications of such transfers? For example, are other marine organisms which man utilizes as food sources capable of transferring dinoflagellate toxins? What are the potential impacts to marine mammal populations, such as seals and otters which consume shellfish, or to whales which graze on various forms of plankton? Where, and how, are the toxins stored within the vectoring organisms, and are the toxins biologically magnified as a result of transfers between trophic levels?

SECTION VI

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Appendix A

MAPS

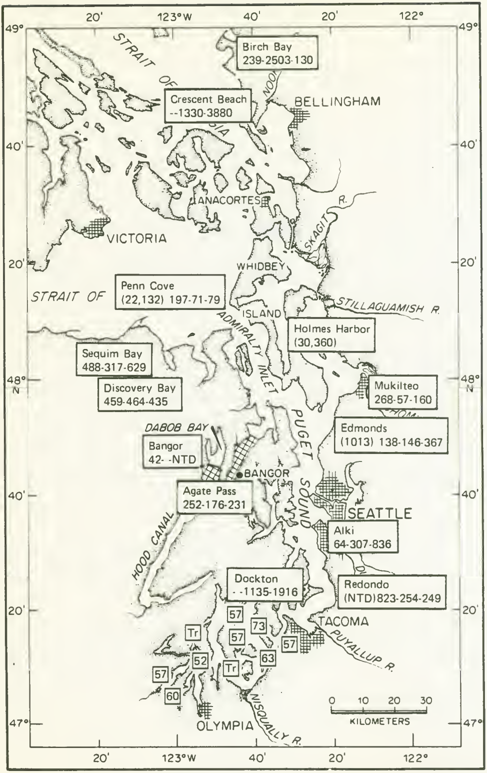

Figure A-1. Highest toxin levels reported at selected sites for 1979-1980-1981. For some stations, 1978 data is given in parentheses. Data for Southern Basin sites is for 1981 only. Toxin levels in µg toxin/100 g shellfish meat. Tr = trace, NTD = no toxin detectable. (Map courtesy of Louisa Nishitani, University of Washington)

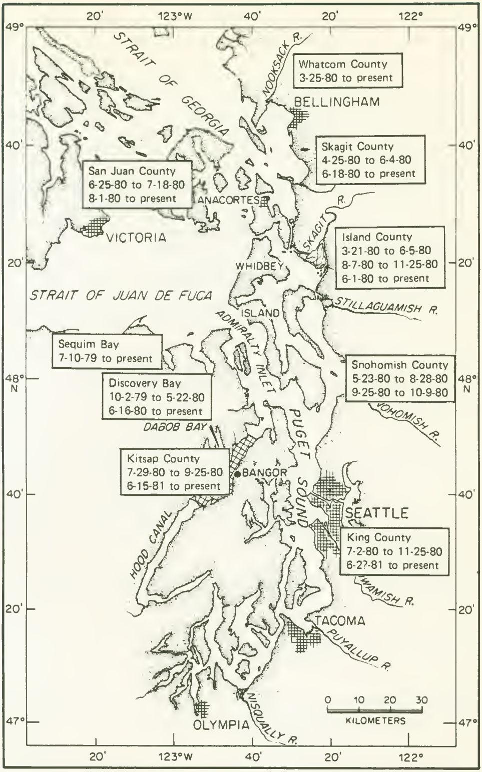

Figure A-2. Distribution of paralytic shellfish poison in inland waters of Washington. Time spans indicate periods in 1980-1981 when harvest of one or more species of shellfish was closed in part or all of the county (bay). Present = 10-1-81. Closure data was provided by Jack Lilja, Department of Social and Health Services. (Map courtesy of Louisa Nishitani, University of Washington)

Appendix B

Glossary

GLOSSARY

Advection:	The process of transport of water, or of an aqueous property, solely by mass motion of the oceans, most typically via horizontal currents.
Autotrophic:	An organism capable of synthesizing organic nutrients directly from simple inorganic substances.
Chelators:	An organic compound in which atoms form more than one coordinate. Bonds with metals in solution.
Depuration:	To free from impurities or heterogeneous matter.
Dinoflagellate:	A member of the algal phylum, Pyrrophata. Most members are biflagellate, unicellular, and planktonic; many members have fixed shapes determined by thick covering plates.
Encystment:	The process of forming or becoming enclosed in a cyst or capsule.
Excystment:	To emerge from a cyst.
Heterotrophic:	An organism that obtains nourishment from the ingestion and breakdown of organic matter.
Hypnocyst:	Resting cyst, a thick-walled, dormant, over-wintering form of cyst.
Macerate:	To cause solid matter to become soft or separated by steeping or mechanical devices.

Melanin:	Any of a group of brown or black pigments occurring in plants and animals.
Motile:	Capable of spontaneous movement.
Phytoplankters:	Passively floating or weakly motile aquatic planktonic plant life.
Vernal:	Occurring in the spring.
Zygote:	A cell produced by the union of two gametes.

Appendix C

References

REFERENCES

Author(s): AOAC Methods
Year: 1980
Title: Paralytic Shellfish Poison, Biological Method

Source: AOAC Methods (on file at Metro library)

Description: The Bioassay method of determining saxitoxin levels of shellfish.

Author(s): Anderson, D.M.
Year: Unknown
Title: Method for Cyst Collection and Processing

Source: Personal communication

Description: A methodology for the collection and processing of sediment samples to be analyzed for the presence of Gonyaulax cysts. This method is not quantitative.

Author(s): Anderson, D.M., Morel, F.M.M.
Year: 1979
Title: The Seeding of Two Red Tide Blooms by the Germination of Benthic *Gonyaulax tamarensis* Hypnocyst.

Source: Estuarine and Coastal Marine Science (1979) 8:279-293

Description: Complementary laboratory and field data are presented that demonstrate the seeding of a spring and a fall bloom of the toxic dinoflagellate *Gonyaulax tamarensis* by the temperature-induced germination of benthic hypnocysts.

Causative Organism: *Gonyaulax tamarensis*

Author(s): Anderson, D.M., Morel, F.M.M.
Year: 1978
Title: Copper Sensitivity of *Gonyaulax Tamarensis*

Source: Limnol. and Oceanogr., 1978, V. 23(2), 283-295

Description: The copper sensitivity of the dinoflagellate, Gonyaulax tamarensis, was examined in artificial seawater medium. Two short term responses of the organism to copper toxicity are rapid loss of motility and reduced photosynthetic carbon fixation. Toxicity to G. tamarensis occurs at the calculated copper activity of natural waters, assuming only inorganic copper complexation. Organic chelation may be necessary before G. tamarensis can successfully compete with other algal species in coastal waters.

Causative Organism: Gonyaulax tamarensis

Author(s): Anderson, D.M., Wall, D.
Year: 1978
Title: Potential Importance of Benthic Cysts of Gonyaulax tamarensis and G. excavata in Initiating Toxic Dinoflagellate Blooms

Source: J. Phycol. 1978, 14(2) pp. 224-234 (on file at Metro library)

Description: Thick walled, nonmotile cyst (termed hypnocyst) of two dinoflagellates were isolated from estaurine sediments in Cape Cod, MA, and germinated to produce their respective motile, thecate stag.

Causative Organism: Gonyaulax tamarensis, G. excavata

Author(s): Ayres, P.A., Cullum, M.
Year: 1978
Title: Paralytic Shellfish Poisoning

Source: Fish. Res. Tech. Rep. Maff Direct. Fish. Res. Lowestoft, 40:23 pp. (on file at Metro library)

Description: A review of the results of annual monitoring of the northeast coast of England from 1968 to 1977, which includes details of some special investigations and concludes with a discussion of the results in light of investigations recorded elsewhere.

Causative Organism: Gonyaulax sp.

Author(s): Ballentine, C.
Year: 1981
Title: Investigators' Report - Red Tide

Source: FDA Consumer Dec. 1980-Jan. 1981 (on file at Metro library)

Description: A brief review of PSP incidents, saxitoxins, and the effect of PSP on humans and on the commercial shellfish industry.

Author(s): Bates, H.A., Rapoport, H.
Year: 1975
Title: A Chemical Assay for Saxitoxin, the Paralytic Shellfish Poison

Source: Agriculture and Food Chemistry Mar/Apr 1975, Vol. 23, No. 2, Page 237 (on file at Metro library)

Description: A chemical assay for saxitoxin has been developed. This chemical assay is 100 times more sensitive than the existing bioassay, and eliminates various problems associated with the bioassay -- particularly at low levels of toxin.

Author(s): Blasco, D.
Year: 1977
Title: Red Tide in the Upwelling Regions of Baja, California

Source: Limnol, Oceanogr. 1977, 22(2): 255-263 (on file at Metro library)

Description: Predominant organisms in a red water dinoflagellate bloom during the seasonal onset of coastal upwelling off Baja, California were Gonyaulax polyedra, Ceratium furca and Gymnodinium sp., with the major part of the chlorophyll and the Sup-14.C assimilation in the area related to the G. polyedra population.

Author(s): Blogoslawski, W.J., Stewart, M.E., Hurst, J.W., Kern, F.G.
Year: 1979
Title: Ozone Detoxification of Paralytic Shellfish Poison in the Softshell Clam (Mya arenaria)

Source: Toxicon, 1979, 17(6): 650-654 (on file at Metro library)

Description: Ozone, <1.2 ppm, was able to inactivate PSP in M. arenaria at levels of <260 g/100g meat, without causing notable damage to the shellfish themselves.

Author(s): Cardwell, R.D., Olsen, S., Carr, M.I., Sanborn, E.W.
Year: 1979
Title: Causes of Oyster Larvae Mortality in South Puget Sound

Source: NOAA Technical Memorandum ERL MESA-39 (on file at Metro library)

Description: Water samples were collected from the southern Puget Sound basin in September, 1977, and characterized for acute toxicity to Pacific Oyster larvae (Crassostrea gigas) chemical composition, and biological composition. Certain receiving waters containing the dinoflagellates Ceratium fusus and Gymnodinium splendens were also tested specifically to determine if they were toxic to oyster larvae. Toxicity tests of two sewage treatment plant effluents, ammonium chloride, and salinity were also conducted.

Author(s): Chew, K.K., Weller, C., Porter, R.G., Beyer, D., Holland, D., Jones, C., Alidina, A., Anderson, R.C., Gustus, R., Weinmann, F.
Year: 1971
Title: Preliminary Survey of Invertebrates and Algae Along the Intertidal Beaches of West Point, the Site of Metro's Sewage Treatment Plant, Seattle, Washington

Source: Metro report

Description: This report represents information gathered from a survey of the major types of macrofauna and macroflora on the north and south intertidal beaches of Metro's West Point sewage treatment plant during late April and early May, 1971.

Author(s): Chew, K.K.
Year: 1973
Title: A Second Survey of Invertebrates and Algae Along the Intertidal Beaches of West Point, the Site of Metro's Sewage Treatment Plant, Seattle, Washington

Source: Metro report

Description: This report presents information gathered from a second survey of the major types of macrofauna and macroflora on the north and south intertidal beaches of Metro's West Point sewage treatment plant during late April and early May, 1973.

Author(s): Collins, M.
Year: 1978
Title: Algal Toxins

Source: Microbiological Reviews, Dec. 1978, pp. 725-746 (on file at Metro library)

Description: A review of the toxins produced by algae. Only toxicity to multicellular organisms was considered.

Author(s): Dale, B., Hurst, J.W., Yentsch, C.M.
Year: 1978
Title: Toxicity in Resting Cysts of the Red Tide Dinoflagellate _Gonyaulax excavata_ from Deeper Water Coastal Sediments

Source: Science 1978, 201, No. 4362: 1223-1225 (on file at Metro library)

Description: A survey of _Gonyaulax excavata_ cyst distribution in bottom sediments, along the Maine coast, found approximately 10 times the toxicity in cysts from 90 m than in motile stages of the same organism.

Causative Organism: _Gonyaulax excavata_

Author(s): Dale, B., Yentsch, C.M.
Year: 1978
Title: Red Tide and Paralytic Shellfish Poisoning

Source: Oceanus 1978, 21(3), 41-49 (on file at Metro library)

Description: This article gives a brief history of PSP outbreaks and the causative organisms. A simplified life history of Gonyaulax sp. is given, along with information on saxitoxin, the PSP poison, and bioassay techniques. The main area of discussion is the east coast of North America.

Author(s): Halstead, Bruce W.
Year: 1965, Revised 1978
Title: Biology of Dinoflagellates and Relationship to Toxic Shellfish

Source: Poisonous and Venomous Marine Animals of the World (dinoflagellate section on file at Metro library)

Description: An overview of toxic shellfish including: biology, history, mechanism of introduction, pathology, public health aspects, ecology, toxicology, and chemistry.

Causative Organism: Gonyaulax sp.

Author(s): Hockey, P.A.R., Cooper, J.
Year: 1980
Title: Paralytic Shellfish Poisoning - A Controlling Factor in Black Oyster Catcher Populations?

Source: Ostrich 1980, 51(3): 188-190 (on file at Metro library)

Description: Mortality of Haemotopus moquini from Red Tide neurotoxin is reported from Saldanha Bay, South Africa, in May, 1979. A rapid succession of outbreaks may have serious consequences for the species, which is already in need of conservation.

Causative Organism: Gonyaulax catenella

Author(s): Hudgins, S.
Year: 1981
Title: New Threats from PSP

Source: Sea Grant Today, May/June, 1981, Vol. II, No. 3, 14-16 (on file at Metro library)

Description: California's PSP Problems.

Causative Organism: <u>Gymnodinium catenatum</u>

Author(s): Hurst, Jr., J.W.
Year: 1979
Title: Shellfish Monitoring in Maine

Source: Toxic dinoflagellate blooms (Taylor/Seliger, Eds.) 1979, 231-234 (on file at Metro library)

Description: A review of Maine's PSP monitoring program; its successes and failures.

Causative Organism: <u>Gonyaulax tamarensis</u>

Author(s): Hurst, Jr., J.W.
Year: 1981
Title: Paralytic Shellfish, PSP, Stations on the Maine Coast

Source: Unpublished (on file at Metro library)

Description: A list of paralytic shellfish stations on the Maine coast for the year 1981.

Causative Organism: <u>Gonyaulax tamarensis</u>

Author(s): Hurst, Jr., J.W., Gilfillan, E.S.
Year: 1977
Title: Paralytic Shellfish Poisoning in Maine

Source: Microbiology Task Force, 10th National Shellfish Sanitation Workshop (on file at Metro library)

Description: Maine has developed a monitoring plan for the detection of PSP which allows for maximum public health protection and commercial use. Rates of toxification and detoxification of various species of shellfish is used to predict the maximum levels that the shellfish will reach and the expected period the shellfish will remain toxic.

Causative Organism: <u>Gonyaulax tamarensis</u>

Author(s): Hurst, Jr., J.W., Gilfillan, E.S.
Year: 1977
Title: Paralytic Shellfish Poisoning on the Maine Coast

Source: Department of Marine Resources Fisheries Circular #30, State of Maine (on file at Metro library)

Description: A description of PSP on the Maine coast.

Causative Organism: Gonyaulax tamarensis

Author(s): Hurst, Jr., J.W., Yentsch, C.M.
Year: 1981
Title: Patterns of Intoxication of Shellfish in the Gulf of Maine Coastal Waters

Source: Canadian Journal of Fisheries and Aquatic Sciences, Volume 38, No. 2, 1981, 152-156 (on file at Metro library)

Description: There is considerable variability of intoxication in Mytilus edulis along the coast of Maine. Patterns noticed are:

1. There are areas of high and low probability of shellfish toxicity.
2. At high probability areas, the timing at different stations is similar.
3. Toxin rise can occur at any time during vernal warming.
4. Toxin levels at offshore stations are frequently higher than toxin levels at nearshore and inshore stations.

Causative Organism: Gonyaulax tamarensis

Author(s): Loeblich, L.A., Loeblich III, A.R.
Year: 1975
Title: Bibliography, First International Conference of Toxic Dinoflagellate Blooms

Source: Bibliography on file at Metro library

Description: A compilation of bibliographies from the First International Conference of Toxic Dinoflagellate Blooms.

Author(s): MacIsaac, J.J., Grunseich, G.S., Glover, H.E., Yentsch, C.M.
Year: 1979
Title: Light and Nutrient Limitation in Gonyaulax excavata: Nitrogen and Carbon Trace Results

Source: Toxic Dinoflagellate Blooms (Taylor/Seliger Eds.), Elsevier North Holland, Inc., Publishers, 1979, 107-110 (on file at Metro library)

Description: The results of some uptake measurements done with 15 N-labelled nitrate and ammonium are present for the dinoflagellate Gonyaulax excavata. Short-term pattern of carbon assimilation into photosynthetic products was measured also.

Causative Organism: Gonyaulax excavata

Author(s): Neal, Richard A.
Year: 1966
Title: Fluctuations in the Level of Paralytic Shellfish Toxin in Four Species of Lamellibranch Molluscs Near Ketchikan, AK, 1963-1965

Source: Thesis Abstract

Description: Correlation between PSP increases and the occurence of G. catenella

Causative Organism: Gonyaulax catenella

Author(s): Neve', R.A.
Year: 1979
Title: Paralytic Shellfish Poisoning in Alaska Waters

Source: Grant Application, Dept. Health, Education and Welfare (on file at Metro library)

Description: The major objective of this proposal is the formulation of a complete description of PSP in Alaska waters.

Causative Organism: Gonyaulax catenella

Newspaper acticles from various sources and authors, all pertaining to PSP worldwide.

Author(s): Nishitani, L., Chew, K.K.
Year: 1979
Title: Investigation of Shellfish Poisoning in Puget Sound

Source: Final report under DHEW/PHS/FDA Order No. FDA-01976-790A (on file at Metro library)

Description: The results of a research program that studied several aspects of the PSP problem in Puget Sound.

Causative Organism: _Gonyaulax catenella_

Author(s): Nishitani, L., Wakeman, J., Chew, K.K.
Year: 1981
Title: Paralytic Shellfish Poisoning Research Program, 1980-1981 University of Washington College of Fisheries

Source: Summary prepared by University of Washington College of Fisheries staff (on file at Metro library)

Description: A summary of closure dates and toxin levels in Puget Sound. Distribution of _Gonyaulax catenella_ in south Puget Sound in 1981. Correlations between hydrographic conditions, plankton composition, _G. catenella_ density, and PSP in shellfish. Results from a study on parasite infestation associated with the decline of a _G. catenella_ bloom. The parasite is _Amoebophrya ceratii_.

Causative Organism: _Gonyaulax catenella_

Author(s): Norris, L., Chew, K.K., Duxbury, A.C.
Year: 1973
Title: Shellfish and the Red Tide

Source: Washington Sea Grant Program WSG-A F 73-2 Also in Pacific Search, June, 1973 (on file at Metro library)

Description: A review of "Red Tide" and PSP in the Pacific Northwest.

Causative Organism: _Gonyaulax catenella_

Author(s): Perry, H.M., Stuck, K.C., Howse, H.D.
Year: 1979
Title: First Record of a Bloom of Gonyaulax monilata in Coastal Waters of Mississippi

Source: Gulf Research Reports 1979, Vol. 6, No. 3: 313-316 (on file at Metro library)

Description: Data are presented on the first recorded bloom of the toxic dinoflagellate Gonyaulax monilata in coastal waters of Florida, Alabama, Mississippi and Louisiana.

Causative Organism: Gonyaulax monilata

Author(s): Prakash, A., Rashid, M.A.
Year: 1968
Title: Influence of Humic Substances on the Growth of Marine Phytoplankton: Dinoflagellates

Source: Limnol. Oceanogr. 1968, B, 598-606

Description: Humic substances, in small amounts, exert a stimulatory effect on marine dinoflagellates that is reflected in increased yield, growth rate, and ^{14}C uptake. The positive effect of humic substances on phytoplankton growth is independent of nutrient concentration and not entirely attributed to chelation processes. Growth enhancement is linked with stimulation of algal cell metabolism. Because of high concentration in coastal waters, humic substances may be regarded as an ecologically signifcant entity influencing phytoplanktonic production.

Author(s): Quayle, D.B.
Year: 1969
Title: Paralytic Shellfish Poisoning in British Columbia, Canada

Source: Fisheries Research Board of Canada 1969, Bulletin 168

Description: This bulletin contains information on PSP in British Columbia. Types of shellfish poisoning are described, and assay procedures and chemistry of the poison are dealt with briefly. The relationships between poisonous shellfish and the causitive organism, and possible means of detoxifying are discussed. Control measures are reviewed.

Causative Organism: Gonyaulax catenella

Author(s): Reid, P.C.
Year: 1980
Title: Toxic Dinoflagellates and Tidal Power Generation in the Bay of Fundy, Canada

Source: Marine Pollution Bulletin (Canada) 1980, II, No. 2: 47-51 (on file at Metro library)

Description: This article indicates the need for future studies to determine if a proposed tidal power station in the Bay of Fundy would alter the water circulation patterns causing a change in the occurence and intensity of PSP.

Causative Organisms: *Gonyaulax excavata*
Gonyaulax tamarensis

Author(s): Ross, N.W.
Year: Unknown
Title: Red Tide - Fact Sheet P814

Source: University of Rhode Island Marine Advisory Service/NOAA Sea Grant (on file at Metro library)

Description: A brief review of PSP information, mainly the east coast of the USA.

Causative Organisms: *Gonyaulax tamarensis*
Gonyaulax excavata

Author(s): Sample, T.E., Saunders, S.L.
Year: 1977
Title: Investigation of Possible Causative Environmental Factors Associated with Toxic Dinoflagellate Blooms

Source: Unpublished (on file at Metro library)

Description: This study investigated what role various physical and chemical parameters play in the occurrence of toxic dinoflagellate blooms. The parameters studied were: surface water temperature, dissolved oxygen, salinity, turbidity, pH, nitrate and nitrite nitrogen, total and orthophosphate, silicate, and ammonia.

Causative Organism: *Gonyaulax catenella*

Author(s): Schmidt, R.J., Loeblich, A.R.
Year: 1979
Title: Distribution of Paralytic Shellfish Poison Among Pyrrhophyta

Source: Journal of the Marine Biological Association (USA), 1979, 59 No. 2: 479-487 (on file at Metro library)

Description: Ten species of the dinoflagellate genus Gonyaulax sp. were investigated to determine the extent and distribution of PSP.

Author(s): Sunda, W.G., Gillespie, P.A.
Year: 1979
Title: The Response of a Marine Bacterium to Cupric Ion and its Use to Estimate Cupric Ion Activity in Seawater.

Source: Journal of Marine Research, 1979, 37(4), 761-777

Description: Experiments were conducted to determine the relationship between the response of a bacterial isolate to copper, as measured by cellular incorporation of ^{14}C-glucose, and the complexation of copper by organic ligands. Inhibition of glucose incorporation was dependent on the cupric ion activity, and was independent of the concentration of organic complexes of copper. At an ambient copper concentration of 0.014 u M (0.9 ppb), cupric ion activity of estuarine seawater was estimated to be $\leq 10^{-11}$M.

Author(s): Various abstracts from authors, all pertaining to RED TIDES

Author(s): White, A.W.
Year: 1980
Title: Recurrence of Kills of Atlantic Herring (Clupea harengus harengus) Caused by Dinoflagellate Toxins Transferred Through Herbivorous Zooplankton

Source: Can. J. Fish and Aquat, Sci. 1980, 37(12): 2262-2265 (on file at Metro library)

Description: G. excavata toxins can, and do, cause herring kills in nature with plankton herbivores, E. nordmanni in this case, acting as a vector. The toxin transfer mechanism is a general phenomenon among herbivorous zooplankton.

Causative Organism: Gonyaulax excavata

Author(s): White, A.W.
Year: 1981
Title: Marine Zooplankton Can Accumulate and Retain Dinoflagellate Toxins and Cause Fish Kills

Source: Limnol. Oceanogr. 1981 26(1), 103-109 (on file at Metro Library)

Description: Herbivorous marine plankton were fed cultures of the toxic dinoflagellate Gonyaulax excavata. Results indicate that there is a fairly general mechanism for the transmission of G. excavata toxins through herbivorous zooplankters to animals at a higher trophic level, and that G. excavata toxins can reach sufficient levels in zooplankters to cause fish kills.

Causative Organism: Gonyaulax excavata

Author(s): Yentsch, C.M., Glover, H.
Year: 1978
Title: Progress Towards an Environmental Predictive Index for Toxic Dinoflagellate Blooms

Source: Tenth Annual Shellfish Sanitation Workshop, Wilt, D.S., ed. 78 (on file at Metro library)

Description: This study addresses dinoflagellate blooms accompanied by measurements of metal activity in solution, and the interaction of metals and chelators in seawater.

Causative Organism: Gonyaulax excavata

Author(s): Yentsch, C.M., Dale, B., Hurst, J.W.
Year: 1978
Title: Coexistence of Toxic and Nontoxic Dinoflagellates Resembling Gonyaulax tamarensis in New England Coastal Waters (NW Atlantic)

Source: J. Phycol. 1978 #14: 330-332 (on file at Metro library)

Description: An attempt to differentiate between toxic and nontoxic dinoflagellates that appear the same under microscopic examination.

Causative organism: Gonyaulax tamarensis